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Research paper

Acclimation to higher VPD and temperature minimized negative effects on assimilation and grain yield of wheat

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ABSTRACT

Adapting to climate change and minimizing its negative impact on crop production requires detailed understanding of the direct and indirect effects of different climate variables (i.e. temperature, VPD). We investigated the direct (via heat stress) and indirect effects (through increased VPD) of high temperature on growth, physiology and yield of two wheat cultivars (Taifun and Vinjett) at two watering levels; well-watered: WW (100% evapotranspiration (ET)) and drought stress: DS (50% of WW ET). Three climate treatments were applied for five days, starting at one week after anthesis. Treatments included hot humid (HH: 36° C; 1.96 kPa VPD), hot dry (HD: 36° C; 3.92 kPa VPD) and normal (NC: 24° C; 1.49 kPa VPD). Difference between HH and HD was considered as the indirect effect of temperature through increased VPD. HD increased transpiration by 2–22% and decreased photosynthetic water-use efficiency (WUE_p) by 24–64% over HH during stress but whole-plant WUE at final harvest was not affected. HD reduced grainfilling duration (3 days), resulted in relatively lower green leaf area (GLA) after the stress and showed a tendency of lower net assimilation rate during the stress compared to HH. However, yield and yield components were not affected under WW conditions due to two reasons (i) acclimation of the photosynthesis, stomatal conductance and rubisco carboxylation efficiency to high temperature and VPD and (ii) translocation of assimilates from stem/leaf to grains after the stress episode. Five days of high temperature stress alone (HH) reduced GLA, grainfilling duration (5 days) and thousand-grain weight (17%), which ultimately reduced grain yield by 17%. DS mainly affected GLA, grainfilling duration and reduced grain yield by 7% vis-à-vis WW. Two cultivars differed only for GLA (lower for Vinjett under HH) and WUE_p (higher for Vinjett under DS). This indicates that the temperature induced increase in VPD has little effect on growth and yield, if sufficient soil moisture is available, because acclimation and tolerance mechanisms tend to alleviate stress effects. These compensatory mechanisms should also be considered when modelling climate effects on crops. However, heat waves and drought events during sensitive crop developmental stages (i.e. anthesis, grainfilling) are important climate variables that need to be considered for adaption to climate change.

1. Introduction

Global annual mean temperature is expected to rise between 1.5 and 6 °C by the end of this century (IPCC, 2013), which could very well interfere with growth and yield of crops (Asseng et al., 2015). Increased overall climatic variability, i.e. frequency, intensity, timing and duration of certain events, are expected to be the common feature of future climate, and large regions are expected to become exposed to higher frequencies of both extreme heat waves and drought periods (IPCC, 2012; Schar et al., 2004; Trnka et al., 2014). Wheat is one of the most

Abbreviations: A_n, net photosynthesis; CE_i, instantaneous carboxylation efficiency of rubisco; DS, drought stress; GLA, green leaf area; g_s, stomatal conductance; HH, hot humid; HD, hot dry; LWR, leaf weight ratio; NC, normal climate; PAR, photosynthetically active radiation; PM, physiological maturity; QC_i, instantaneous quantum cost; SD, stress-day; SLA, specific leaf area; SPAD, chlorophyll content index; StWR, stem weight ratio; SWR, spike weight ratio; TAGB, total aboveground biomass; T_r, transpiration; VPD, vapor pressure deficit; WUE, water-use efficiency; WUE_{GY}, WUE for grain yield; WUE_i, intrinsic WUE; WUE_p, photosynthetic WUE; WUE_{TY}, WUE for total aboveground biomass; WW, well-watered; Ψ_L, leaf water potential

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sensitive crops to high temperature (Satorre and Slafer, 1999). It may experience heat stress during all phenological stages, but anthesis and grainfilling phases (end-of-season stress or terminal stress) are generally more sensitive (Wollenweber et al., 2003). The terminal heat stress is expected to rise due to changes in climatic variability (Semenov, 2009). The upper optimum temperature limit for wheat during anthesis and grainfilling has been reported to be around 34° C (Farooq et al., 2011), and temperature higher than this seriously reduces grain yield (Ferris et al., 1998).

The effects of higher seasonal mean temperature (Asseng et al., 2015; Porter and Gawith, 1999) and extreme high and variable temperatures (Asseng et al., 2011; Ferris et al., 1998; Wollenweber et al., 2003) on wheat physiology, growth, and other yield determinants have been extensively studied; however, in order to develop climate resilient management practices and crop cultivars, detailed understanding of the actual mechanisms behind these effects is required. The effects of extreme heat waves on some of the yield determinants in wheat viz. accelerated and premature senescence, reduction in grain weight have been documented (Farooq et al., 2011; Vignjevic et al., 2015), but attributing these negative effects exclusively to temperature is not straight forward. This is because of the hidden and confounding effects of vapor pressure deficit (VPD), which increases exponentially with increasing temperature at constant absolute humidity. High VPD amplifies the evaporative power of the surrounding environment and serves as the major driving force behind evaporative and transpirational water losses. Certainly, it is not possible to deny the direct heat effects, but high temperatures also acts indirectly by changing plant water-use and intensifying drought effects through higher VPD (Will et al., 2013). A comprehensive meta-analysis study by Gourdji et al. (2013) revealed that heat tolerance in wheat is influenced by changes in daily VPD. Under extreme heat stress, VPD not only increases plant water demand due to high transpiration rates but also reduces the future soil water supply (Lobell et al., 2013), and it thereby acts as dual stress. However, this notion cannot be taken as absolute because of the fact that VPD driven high transpiration can also serve as a negative feedback by lowering crop canopy temperature and consequently VPD is reduced, which ultimately may mitigate heat stress (Gourdji et al., 2013).

The effect of heat stress through enhanced VPD also depends on plant stomatal behavior which is crucial for regulating leaf gas exchange (H_2O loss and CO_2 influx). Crop species or cultivars may differ in their stomatal response to VPD and drought (Schoppach and Sadok, 2012), which leads to different water-use efficiency (CO_2 uptake vs. H_2O loss). Cultivars showing little control over water loss are termed as anisohydric (McDowell et al., 2008) as revealed for peanut genotypes (Devi et al., 2009). These cultivars could be ideal if soil water is not a limiting factor. In contrast, isohydric cultivars show conservative behavior and tend to close stomata in order to maintain plant water status (McDowell et al., 2008) as observed for pearl millet (Kholová et al., 2010) and chickpea (Zaman-Allah et al., 2011). Nonetheless, irrespective of plant stomatal behavior, high VPD may amplify the physiological stress under water limiting conditions, either by reduced CO_2 influx (isohydric) or by enhanced transpirational water loss (anisohydric) (McDowell et al., 2008). Thus, cultivars with isohydric nature may exhibit higher water-use efficiency under stress conditions, but at an expense in terms of total biomass production. However, suitability of these two groups of cultivars also depends on timing and duration of drought. Generally, isohydric cultivars are suggested to be more suitable under terminal drought while anisohydric under intermittent drought (Polania et al., 2016).

Heat and drought stresses are, therefore, interlinked through VPD with the two factors reinforcing each other. Previous model-based analyses on maize (Lobell et al., 2013) showed that a significant proportion of the heat effects may be attributed to indirect effects of changes in VPD, which modulates plant water use and carbon assimilation. However, models differ in their description of crop responses to temperature and VPD, and they mostly do not account for acclimation responses to changes in these conditions. Therefore, despite ongoing efforts to improve the modelling of crop responses to increased temperatures and drought, large uncertainties still remain (Asseng et al., 2015; Cammarano et al., 2016). Other studies have suggested a positive impact of VPD on crop yield, and associated this with higher solar radiation on days with high VPD (Lobell, 2007; Roberts et al., 2012). Experimental work by Will et al. (2013) on forest species, Schoppach and Sadok (2012); Schoppach and Sadok (2013) on wheat and Yang et al. (2012) on maize considered the effect of VPD, but they focused mainly on transpiration. Furthermore, extreme heat was not in focus in either of these studies.

Nonetheless, due to contrasting results and lack of experimental evidence, VPD effects on grain yield of wheat at high temperature need to be better understood. We therefore aimed to investigate the combined effects of high temperature and high VPD on growth and yield of two wheat cultivars at two watering levels. We hypothesize that, as a consequence of high temperature, higher VPD will reduce grain yield by increasing crop water demand, restricting CO_2 assimilation, hastening leaf senescence and crop maturity. Since at high temperature plants can suffer from heat as well as high VPD, we further hypothesize that the effects of high temperature and high VPD will be additive and more severe than the temperature effects alone. The objectives of this study were (1) to estimate the effects of post-anthesis heat wave at low and high VPD on growth, physiology and yield of two wheat cultivars under drought and well-watered conditions, and (2) to quantify the effects of high VPD on growth and physiology of wheat, and to what extent these effects actually translate into yield loss.

2. Materials and methods

2.1. Experiment introduction

This experiment was designed to serve three purposes (1) to study the effects of post-anthesis heat wave on growth and physiology of wheat thus a high temperature treatment with low VPD was designed to be applied for five days starting from six days after the mid-flowering stage (GS65) (2) To separate the direct (through heat stress) and indirect (through increased VPD) effects of high temperature, therefore a second high temperature treatment was designed with high VPD. A third treatment with normal temperature- and VPD was included as a control. Temperature and VPD treatments collectively were termed as “climate treatments” (3) the above mentioned climate treatments were meant to be studied under well-watered and drought stress conditions for two cultivars (see Table 1 and section 2.3 for treatment details).

2.2. Plant material and cultivation

A controlled environment experiment was conducted in growth chambers at Research Centre Foulum, Aarhus University, Denmark. Thirty seeds of two wheat cultivars of Scandinavian origin, namely *Taifun* and *Vinjett*, were sown at 2.5–3 cm depth in plastic pots (cap.

Table 1
Set points for climate treatments in climate chambers.

Chamber	Treatment notation	Time	T (°C)	RH (%)	VPD (kPa)	CO_2 (ppm)
I	Normal climate (NC)	day	24	50	1.49	400
		night	12	76	0.34	400
II	Hot humid climate (HH)	day	36	67	1.96	400
		night	26	90	0.34	400
III	Hot dry climate (HD)	day	36	34	3.92	400
		night	26	80	0.67	400

T, temperature; RH, relative humidity; VPD, vapor pressure deficit; NM, HH and HD are the notations for treatments in text.

10 l) filled with 9 kg mixture (1:1 v/v) of sandy soil (sand 68%, silt 21%: clay 8%, humus 2.7% subsoil from Jyndevad, Denmark) and sphagnum peat (substrate no. 4, pH 5.6-6.4, size 10–30 mm, Pindstrup Mosebrug A/S, Denmark). The experiment was conducted from October 2015 to February 2016. After sowing (13 Oct) the pots were kept in the greenhouse until emergence and later on transferred to the climate chambers where typical Danish growing season April to August climate for spring wheat was maintained (Fig. 1S). Non-experimental pots with plants and thus completely similar to the experimental pots were maintained to estimate the irrigation water requirements during the stress treatments as described later. Three weeks after sowing, thinning was done to obtain the desired density of 20 seedlings per pot.

Field capacity ca. 30% v/v and wilting point ca. 7% v/v of the soil–peat mixture were estimated before sowing. No drought stress was allowed throughout the growing season, except during drought treatments; described below. Water was applied either by weighing the pots after stratified random selection or on the basis of soil water content measurements with time domain reflectometry (TDR-100, Campbell Scientific, UT, USA) for which two steel rods of 17 cm length were installed in the center of each pot. Total amount of water used per pot at the time of final harvest (evapotranspiration; ET) was considered equivalent of total amount of irrigation plus the soil water in pots before sowing minus the residual after final harvest. Inorganic liquid fertilizer (Prima væksthusgødning, Denmark) containing macro- and micro nutrients was applied in two equal splits 23 and 40 days after sowing, with 200:34:158 kg NPK ha^{-1} and other complementary nutrients including Mg (0.01% + additional dose of $MgSO_4$), Cu (0.005%), Zn (0.0025%), B (0.01%), Mo (0.001%) and Mn (0.01%). Plants were sprayed with fungicides against powdery mildew.

2.3. Treatments

Plant developmental stages were determined and noted regularly according to the BBCH scale (Lancashire et al., 1991). Both cultivars reached the anthesis stage on the same day (5th Jan). Six days after anthesis (mid-flowering − GS65), pots were divided in three groups and placed in three separate climate chambers for stress treatments. The walk-in climate chambers were identical with internal dimensions of 2.40 × 1.97 × 2.05 m (width, length, height) and had precise control over a wide range of relative humidity, temperature, light intensity and CO_2 concentration (MB-Teknik, Brøndby, Denmark). Three different climates (climate treatments) viz. normal (NC), hot humid (HH) and hot dry (HD) were set in three chambers (Table 1). The only difference between the HH and HD was VPD, which was doubled (1.96–3.92 kPa) in the latter by decreasing relative humidity. Light intensity (PAR) was set to be 1000 µmol m^{-2} s^{-1} at canopy height during the day. The durations of day and night were 16 and 8 h, respectively. Temperature, relative humidity and PAR were ramped up and down uniformly in steps during the transition periods of day and night; however, these variables were kept constant from 9am to 5pm at the exact levels as shown in Table 1.

Two irrigation regimes were included in the experiment; well-watered (WW) where 100% of evapotranspiration (ET) was replaced by irrigation, and drought stress (DS) in which water application was 50% of what applied to the WW plants. The ET under different climate settings was estimated before the actual treatments by incubating WW non-experimental pots (exactly similar to the experimental pots) in the chambers with pre-programmed settings (Table 1).Total weight loss (ET) of a pot in one day was taken as the water requirement for WW treatment for a given climate. Estimated amount of water required for three climatic conditions viz. normal, hot humid and hot dry for WW treatment was ca. 1000, 1500 and 2000 ml, respectively. For DS treatment, pots were brought to 50% FC (ca. 15% v/v) on the first morning of stress period, and later on water application was 50% of what applied to the WW plants. In order to reach 50% FC (ca. 15% v/v and 15% of pot volume) for DS treatment, water application was withheld on the day before the treatments started. Both for WW and DS, water was applied in the morning through a drip irrigation system. Temporary drip irrigation system was installed to make sure that pots get uniform and continuous supply of water during the day, which helped to take measurements at same moisture level (see Fig. 2S for details of irrigation system). It should be noticed that the water application was based on the estimated ET under different climate settings (Table 1) before the actual treatments and it was assumed that the ET will not change over the stress period. Pots were placed on trolleys (2 pots per trolley) and trolleys were regularly repositioned during the experiment. Climate and irrigation treatments were implemented for both cultivars and lasted for five days.

2.4. Measurements

Flag leaves for the measurements of gas exchange, chlorophyll content index (SPAD) and chlorophyll fluorescence were tagged prior to start of treatments. Leaf gas exchange parameters, i.e. stomatal conductance (g_s), transpiration (T_r) and net photosynthesis (A_n), were measured on first and third day of stress (stress day 1 and 3 (SD, hereafter)). Gas exchange measurements were carried out between 10:00 and 14:00 h on fully expanded flag leaves from center of the pots (n = 3) with CIRAS-2 portable photosynthesis system (PP system Inc. MA, USA) at a light intensity of 1500 µmol m^{-2} s^{-1}. The cuvette environment (temperature, VPD and CO_2) was set to match the outside environment during measurements. Flow rate in the cuvette was 250 µmol m^{-2} s^{-1}. Photosynthetic and intrinsic water-use efficiencies (WUE_p & WUE_i respectively, hereafter) were defined and calculated as A_n/T_r and A_n/g_s. The instantaneous quantum cost of carbon assimilation (QC_i) and chlorophyll fluorescence parameter F_v/F_m (maximum quantum yield of PSII) were measured following Jiao et al. (2017). Instantaneous carboxylation efficiency of CO_2 assimilation (CE_i) was calculated as A_n/C_i. Flag leaf temperature was measured on SD3 from the center of leaves (n = 8) with the help of an infrared thermometer (accuracy ± 2%; Probuilder model no. 9320, Denmark) between 12:00-13:00 h. Chlorophyll content index (SPAD) was measured on SD3 from the center of flag leaves (n = 6)) using a SPAD meter (Hydro, Japan) between 9 and 10 am. Midday (12:00- 14:00) leaf water potential of flag leaves was measured with a pressure chamber (Soil Moisture Corp, Santa Barbara, CA) on stress day 5 (SD5).

Three days after the stress period, 40 plants per treatment were harvested for assessment of total biomass, biomass of different plant organs and leaf area. Plants from each pot were separated into stems, spikes and leaves. Green leaf area (GLA) was measured using LI-3100 (LI-COR, Lincoln, Nebraska, USA). Leaves more than 50% yellow were not considered in GLA measurements. All plant parts (leaves, spikes and stems) were oven dried separately at 60 °C for 72 h. Specific leaf area (SLA) was calculated as the ratio of leaf area (cm^2) to leaf dry weight (g). Leaf weight ratio (LWR), spike weight ratio (SWR) and stem weight ratio (StWR) were calculated as ratio of dry weight of concerned plant part to total aboveground plant dry matter. Total aboveground biomass (TAGB) was calculated as the sum of dry weights of stems, spikes and leaves. The remaining plants were well managed under normal climate and well-watered conditions until harvest. Before harvesting, time taken to physiological maturity was monitored following the CIMMYT guide (Bell and Fischer, 1994). Harvesting was done (n = 4) when all plant parts were dry and grains had reached maturity. These samples were used to determine total aboveground biomass (TAGB), grain yield, and thousand grain weight (TGW). Whole plant water-use efficiency for grain yield (WUE_{GY}) and total aboveground yield (WUE_{TY}) were calculated as ratio of grain yield and TAGB at final harvest to total water used by the plants which included both evaporation and transpiration.

2.5. Statistical analysis

Before statistical analysis, data on all parameters were tested against

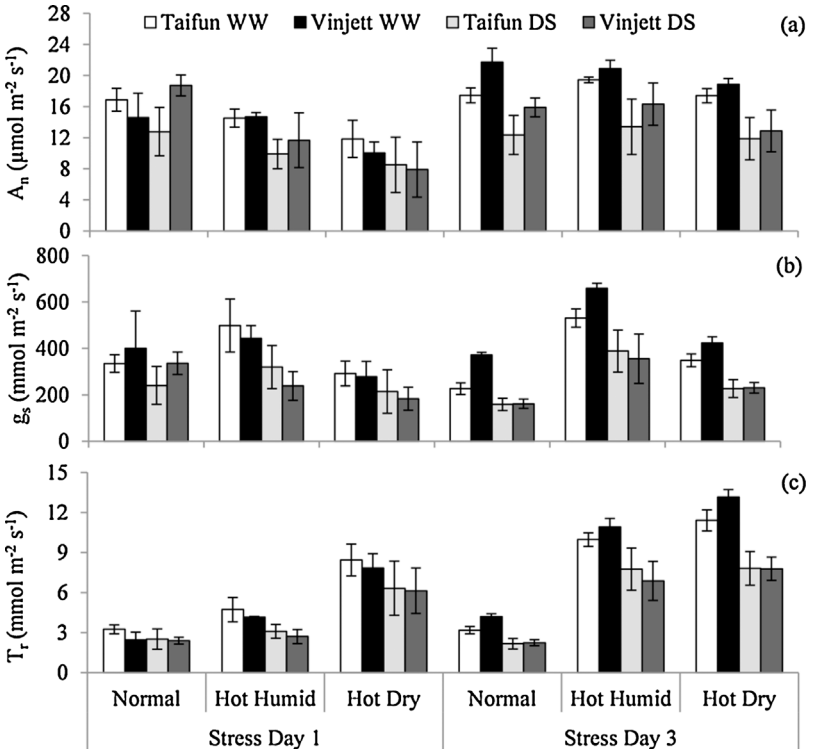

Fig. 1. (a) Net photosynthetic rate (A_n), (b) stomatal conductance (g_s) and (c) transpiration (T_r) of wheat cultivars (Taifun and Vinjett) as affected by irrigation (well-watered, WW; drought stress, DS) and climate (normal: 24° C, 1.49 kPa VPD; hot humid: 36° C, 1.96 kPa VPD; and hot dry: 36° C, 3.92 kPa VPD). Measurements were done on 1st and 3rd day of stress treatments. Error bars represent ± SE (n = 3).

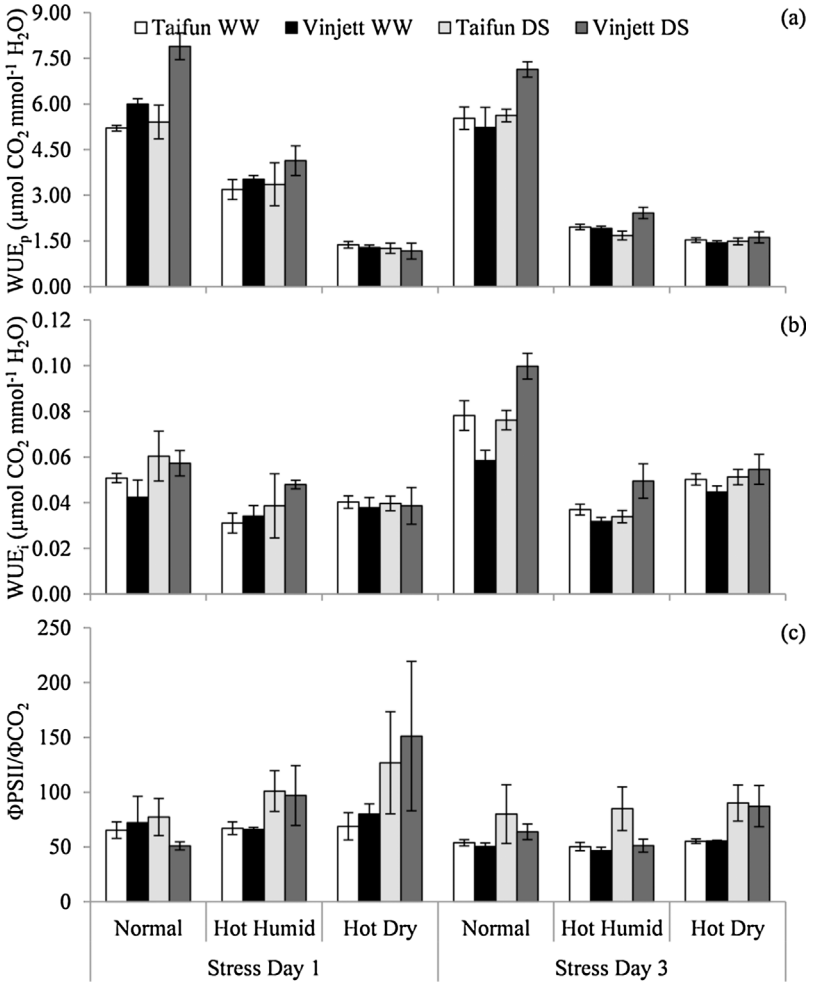

Fig. 2. (a) Photosynthetic water-use efficiency (WUE_p), (b) intrinsic water-use efficiency (WUE_i) and (c) instantaneous quantum cost of assimilation (ΦPSII/ΦCO_2) of wheat cultivars (Taifun and Vinjett) as affected by irrigation (well-watered, WW; drought stress, DS) and climate (normal: 24° C, 1.49 kPa VPD; hot humid: 36° C, 1.96 kPa VPD; and hot dry: 36° C, 3.92 kPa VPD). Measurements were done on 1st and 3rd day of stress treatments. Error bars represent ± SE (n = 3).

Shapiro-Wilk test (Shapiro and Wilk, 1965), followed by log transformation, if needed. Data were subjected to three-way analysis of variance (ANOVA) for effects of climate, irrigation and cultivars, and multiple comparisons of treatment means were performed using Tukeý's HSD test at 5% level of significance. All analysis and tests including the correlation for some variables were performed with Statistix ver. 8.1 (Statistix, Tallahassee, FL, USA). Changes in A_n and g_s from SD1 to SD3 for different climate, cultivar and irrigation treatmetns were analysed with permutation test (Lehmann, 1998).

3. Results

3.1. Leaf gas exchange, water-use efficiency and quantum cost on SD1 and SD3

Results for the effect of extreme heat at low and high VPD and drought on net photosynthesis (A_n), stomatal conductance (g_s) and transpiration (T_r) are presented in Fig. 1. A_n was affected only by climate ($P < 0.05$) on SD1 where it was reduced by 20 and 40% under HH and HD, respectively, compared to normal climate (NC). In contrast on SD3, only irrigation ($P < 0.001$) influenced A_n where DS reduced it by 30%. g_s was affected by irrigation ($P < 0.05$) on SD1 and climate ($P < 0.001$) and irrigation ($P < 0.001$) on SD3. DS reduced g_s by 35 and 43% on SD1 and SD3, respectively. On SD3, g_s was 28 and 53% higher for HH and HD, respectively, compared to NC. T_r was affected by climate and irrigation on both stress days. Transpiration was enhanced by 63 and 28% under HD and HH, respectively, compared to NC on SD1. On SD3, HH and HD had 67–70% more T_r than NC. T_r was 21 and 35% higher from well-watered compared to drought stressed plants on SD1 and SD3, respectively. The analysis for changes in A_n and g_s from SD1 to SD3 for different treatments is shown in Table 1S. There were no significant differences ($P > 0.05$) in these parameters from SD1 to SD3 for NC, except for g_s of Vinjett cultivar under DS. Comparatively, significant changes were observed in A_n and g_s from SD1 to SD3 under WW conditions ($P < 0.05$), both for HH and HD. g_s of Taifun cultivar however remained unchanged.

Results for photosynthetic water-use efficiency ($A_n/T_r = WUE_p$), intrinsic water-use efficiency ($A_n/g_s = WUE_i$), and instantaneous quantum cost (QC_i) are presented in Fig. 2. WUE_p was strongly influenced by climate ($P < 0.001$) on SD1 and by climate ($P < 0.001$) and cultivar × irrigation interaction ($P < 0.01$) on SD3. On SD1, WUE_p was 79 and 42% lower for HD and HH, respectively, compared with NC, with significant difference between the two extreme climates. Likewise on SD3, HD and HH differed significantly and subsequently resulted in 74 and 66% lower WUE_p than NC. The WUE_p under NC and HD did not change from day 1 to day 3 but it declined under HH during this period. Drought stress enhanced the WUE_p, but the effect was observed only for Vinjett cultivar and it disappeared in the HD treatment. WUE_i was significantly lower under DH and HH compared to NC on SD1. On SD3, NC and HH showed the highest and lowest WUE_i, respectively. The DS plants had significantly higher WUE_i on SD3 than WW but the effect was only observed for Vinjett cultivar. The QC_i (defined and calculated as $\Phi PSII/\Phi CO_2$) showed a tendency of higher values when moving from NC to HD and also in drought stressed plants on SD1. However, drought significantly increased QC_i by 28% on SD3 ($P < 0.01$).

3.2. SPAD, F_v/F_m and instantaneous carboxylation efficiency on SD3

Results for SPAD, F_v/F_m and instantaneous carboxylation efficiency ($CE_i = A_n/C_i$; umol m^{-2} s^{-1}/umol mol^{-1}) on SD3 are shown in Fig. 3. Chlorophyll content index (SPAD) was not affected by stress treatments, but Taifun cultivar had significantly higher (ca.10%) SPAD values than Vinjett. F_v/F_m values were significantly higher under NC than the other climate treatments, which did not differ. CE_i on SD3 was significantly (24%) lower in DS treatments.

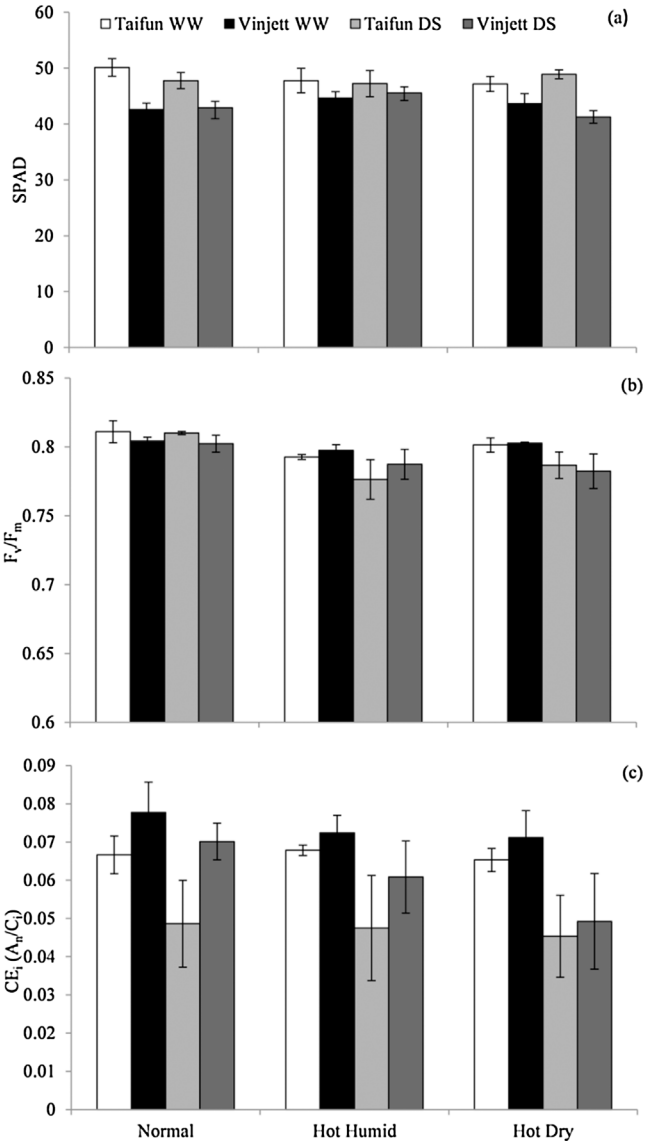

Fig. 3. (a) Chlorophyll content index (SPAD), (b) maximum quantum yield of photosystem II (F_v/F_m) and (c) Instantaneous carboxylation efficiency of rubisco ($CE_i = A_n/C_i$) as affected by irrigation (well-watered, WW; drought stress, DS) and climate (normal: 24° C, 1.49 kPa VPD; hot humid; 36° C, 1.96 kPa VPD; and hot dry: 36° C, 3.92 kPa VPD). Measurements were done on 3rd day of stress treatments. Error bars represent ± SE (n = 3 for CEi and 6 for F_v/F_m and SPAD).

3.3. Leaf temperature on SD3

The flag leaf cooling (air minus leaf temperature) was affected by both climate and irrigation treatments. Highest cooling of the flag leaf was observed under HH (ca. 6.8° C) followed by HD (ca. 5.8° C) (Fig. 4c) but with no significant difference between HH and HD. The cooling of the flag leaf was linearly related to transpiration ($R^2 = 0.87$; $P < 0001$, Fig. 4a) and stomatal conductance ($R^2 = 0.65$; $P < 0.01$) (Fig. 4b).

3.4. Midday leaf water potential on SD5

Midday leaf water potential (Ψ_L) on the last day of stress treatments (SD5) was affected by climate ($P < 0.001$), irrigation ($P < 0.0001$) and their interaction ($P < 0.05$) (Fig. 5). Plants grown under HD showed significantly lower Ψ_L than NC and HH. Likewise, the drought stressed plants had lower Ψ_L than those growing under well-watered conditions. Under well-watered conditions, Ψ_L was not significantly

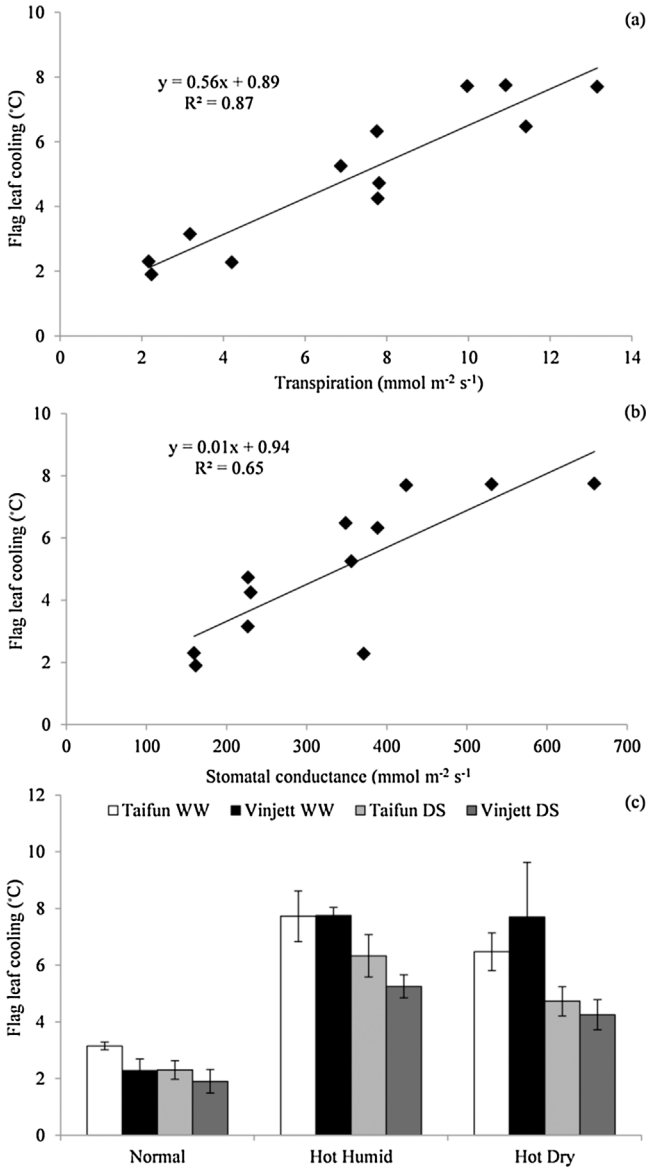

Fig. 4. Flag leaf cooling (air minus leaf temperature) and its relationship with leaf transpiration (a) and stomatal conductance (b) under different irrigation (well-watered, WW; drought stress, DS) and climatic conditions (normal: 24° C, 1.49 kPa VPD; hot humid; 36° C, 1.96 kPa VPD; and hot dry: 36° C, 3.92 kPa VPD). Taifan and Vinjett are two cultivars. Measurements were done on stress day 3. Error bars represent ± SE (n = 8).

different for the three climates (although with a tendency of lower Ψ_L when moving from NC to HD). In contrast, drought stressed plants under HH had significantly higher Ψ_L than under HD. There were no differences among cultivars in these responses.

3.5. Green leaf area, specific leaf area and total dry matter after the stress period

Responses of green leaf area (GLA), total aboveground biomass (TAGB) and specific leaf area (SLA) on day 3 after the stress period are presented in Table 2 and Table 3. GLA was significantly affected by all three experimental factors and climate × cultivar interaction (Table 2). The two extreme climates (HH and HD) reduced GLA compared to NC, but with different responses for the two cultivars. Drought reduced GLA by 20%. The cultivar Taifun had higher GLA than Vinjett, and the cultivars responded differently to climate with the effect of HH being more severe for Vinjett than Taifun. SLA was not affected by the

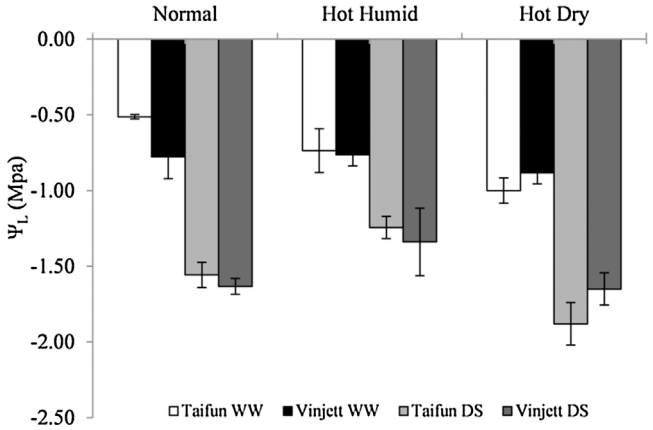

Fig. 5. Midday leaf water potential of wheat cultivars (Taifun and Vinjett) as affected by irrigation (well-watered, WW; drought stress, DS) and climate (normal; 24° C, 1.49 kPa VPD; hot humid; 36° C, 1.96 kPa VPD; and hot dry; 36° C, 3.92 kPa VPD). Leaf water potential was measured on last day of stress treatments (SD5). Error bars represent ± SE (n = 3).

experimental treatments. TAGB showed little consistent effect of experimental treatments, and there was a weak interaction of cultivar × irrigation with reduced TAGB under drought stress for Vinjett, but not for Taifun under HD climate.

3.6. Assimilate allocation after the stress period

The allocation of assimilates to different plant parts (viz. spike, stem and leaves) on third day after the stress period was affected by the experimental treatments (Table 2 and Table 3). Leaf weight ratio (LWR) was affected by all experimental factors. Both extreme climates (HH and HD) as well as drought stress reduced LWR. The cultivars responded differently to climate, showing no difference at HD but with significant cultivar differences under other two climates. The greatest reduction in LWR for Vinjett was observed under HH, while for Taifun the greatest reduction was under HD. The highest spike weight ratio (SWR) was recorded under HD, followed by HH and NC. From the two cultivars, SWR was higher for Taifun than Vinjett. Drought stressed plants accumulated relatively more biomass in grains resulting in significantly higher SWR than well-watered plants. The stem weight ratio (StWR) largely responded to treatments in the opposite way to SWR. The effect of climate on StWR was only seen for Vinjett cultivar where StWR reduced with increasing severity of stress from NC to HD but with no significant difference between HH and HD.

3.7. Yield and yield attributes at final harvest

Data on final grain yield (GY), total aboveground biomass at final harvest (TAGB), thousand grain weight (TGW), days to physiological maturity (PM), WUE_{TY} and WUE_{GY} at maturity are presented in Tables 2 and 3. GY was affected by climate and irrigation treatments. GY was significantly reduced (12 and 17%) under HD and HH, respectively, when compared to NC. Drought stress significantly reduced GY (ca. 7%) against well-watered conditions. TAGB was reduced by 14% in both extreme climates compared with NC. DS reduced TAGB by 9%, but the effect of drought was only observed for Vinjett. TGW was significantly influenced by climate, cultivar and interaction between cultivar and irrigation. TGW was significantly different for all three climates and it was 14 and 17% lower under HD and HH, respectively, when compared to NC. The effect of drought was only observed for Taifun where it reduced TGW by 5%.

Time taken to physiological maturity (PM) by the crop plants was affected by all factors, and the effect of climate differed between cultivars and irrigation regimes. DS reduced the maturity period by 3 days.

Table 2
Output of three-way analysis of variance (ANOVA) for various plant parameters.

Source of variation	Measurements done 3 days after the stress treatments						At final harvest					
	GLA	SLA	TAGB	LWR	SWR	StWR	GY	TGW	TAGB	PM	WUE_{GY}	WUE_{TY}
Climate (C)	***	ns	ns	***	***	***	***	***	***	***	***	***
Cultivar (Cul)	***	ns	ns	***	***	***	ns	***	***	*	ns	***
Irrigation (I)	***	ns	ns	***	*	ns	**	ns	***	***	ns	ns
C × Cul	**	ns	ns	*	ns	*	ns	ns	ns	**	ns	ns
C × I	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Cul × I	ns	ns	*	ns	ns	ns	ns	**	*	ns	ns	ns
C × Cul × I	ns	ns	*	**	ns	ns	ns	*	ns	*	ns	ns

The table shows level of significance for green leaf area (GLA), specific leaf area (SLA), total above ground dry matter (TAGB), leaf weight ratio (LWR), spike weight ratio (SWR), stem weight ratio (StWR), grain yield (GY), thousand grain weight (TGW), total aboveground biomass (TAGB) at final harvest, time taken to physiological maturity (PM), whole plant water use efficiency on the basis of grain yield (WUE_{GY}) and TAGB (WUE_{TY}) as affected by climate, cultivar, irrigation and their interactions. '*', '**' '***' indicates significance level at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, ns is non-significant.

Vinjett matured slightly earlier than Taifun. The maturity period was reduced by 5 and 8 days for HH and HD, respectively, compared with NC. Irrespective of the climate, drought stressed plants matured earlier than WW plants. Drought stressed plants growing under HH matured at the same time as well-watered plants growing under HD. WUE_{TY} was influenced by climate and differed between cultivars. WUE_{TY} for NC was significantly higher than the two extreme climates. The Vinjett cultivar had significantly higher WUE_{TY} than Taifun. WUE_{GY} was only affected by climate where NC exhibited significantly higher WUE than the other two climates.

4. Discussion

4.1. Grain yield, green leaf area and assimilate partitioning

Both, positive (Lobell, 2007; Roberts et al., 2012) and negative (Lobell et al., 2013) impacts of high VPD at high temperature on grain yield have been reported in model-based analytical studies; however, experimental evidences for these relationships are lacking. Our study showed similar effects of high and low VPD under heat stress on grain yields and total aboveground biomass of wheat. Although, high VPD (HD) reduced grainfilling duration by 3 days and resulted in relatively lower post-stress GLA (Table 3), the grain yield was not affected

Table 3
Treatment effects on biomass, water-use efficiency and yield components.

Treatment combinations		3 days after the stress period			At final harvest					
		GLA (cm^2 $plant^{-1}$)	SLA (cm^2 g^{-1})	TAGB (g $plant^{-1}$)	GY (g pot^{-1})	TGW (g)	TAGB (g pot^{-1})	PM (days)	WUE_{GY} (g L^{-1})	WUE_{TY}(g L^{-1})
Normal (N)	Taifun-WW	103.1 ± 12.3	219.6 ± 2.0	7.97 ± 0.7	84.4 ± 3.7	41.7 ± 1.49	243.0 ± 7.2	121.5 ± 0.3	1.66 ± 0.06	4.78 ± 0.14
	Taifun-DS	77.3 ± 4.3	208.9 ± 5.9	9.21 ± 1.0	72.8 ± 4.1	37.9 ± 0.76	221.0 ± 11.1	117.8 ± 0.3	1.55 ± 0.09	4.71 ± 0.26
	Vinjett-WW	79.9 ± 10.9	218.6 ± 10	9.31 ± 0.4	78.6 ± 4.4	36.7 ± 1.02	290.8 ± 8.1	120.8 ± 0.5	1.55 ± 0.08	5.72 ± 0.22
	Vinjett-DS	74.4 ± 3.2	220.3 ± 8.3	8.42 ± 0.3	74.3 ± 2.6	37.6 ± 0.56	246.1 ± 11.6	118.3 ± 0.5	1.61 ± 0.05	5.34 ± 0.23
Hot humid (HH)	Taifun-WW	79.7 ± 8.7	213.4 ± 12.7	8.50 ± 0.3	64.3 ± 2.8	34.8 ± 1.76	196.6 ± 6.1	115.3 ± 0.6	1.22 ± 0.05	3.74 ± 0.13
	Taifun-DS	48.4 ± 4.8	219.8 ± 5.5	8.03 ± 0.6	64.8 ± 1.9	32.3 ± 0.48	192.6 ± 5.2	112.5 ± 0.3	1.30 ± 0.04	3.87 ± 0.11
	Vinjett-WW	71.9 ± 2.6	204.4 ± 19.1	7.69 ± 0.4	66.1 ± 3.4	30.3 ± 0.67	241.0 ± 7.4	116.3 ± 0.3	1.27 ± 0.05	4.62 ± 0.13
	Vinjett-DS	36.9 ± 2.5	231.4 ± 4.3	7.94 ± 0.2	60.3 ± 4.6	31.2 ± 0.23	209.8 ± 10.8	114.8 ± 0.5	1.27 ± 0.09	4.40 ± 0.20
Hot dry (HD)	Taifun-WW	65.3 ± 3.4	205.1 ± 18.4	7.20 ± 0.5	69.1 ± 0.9	35.1 ± 0.52	205.1 ± 4.9	113.3 ± 0.3	1.25 ± 0.01	3.72 ± 0.11
	Taifun-DS	70.5 ± 3.5	191.1 ± 12.4	9.05 ± 0.8	66.0 ± 2.5	33.9 ± 0.77	197.1 ± 7.5	110.0 ± 0.4	1.33 ± 0.06	3.98 ± 0.19
	Vinjett-WW	54.3 ± 1.4	205.9 ± 11.7	8.50 ± 0.1	71.5 ± 3.7	33.4 ± 1.31	232.1 ± 14.2	114.0 ± 0.4	1.30 ± 0.06	4.21 ± 0.23
	Vinjett-DS	42.9 ± 4.5	213.0 ± 5.9	7.65 ± 0.1	66.2 ± 1.1	30.4 ± 0.44	204.9 ± 3.0	109.5 ± 0.3	1.33 ± 0.02	4.12 ± 0.04
Main effects										
Climate	Normal	83.7 A	216.8	8.73	77.5 A	38.5 A	250.2 A	119.6 A	1.59 A	5.14 A
	Hot humid	59.2 B	217.2	8.04	63.9 B	32.1 B	210.0 B	114.7 B	1.26 B	4.16 B
	Hot dry	58.3 B	203.8	8.09	68.2 B	33.2C	209.8 B	111.7C	1.30 B	4.0 B
Cultivars	Taifun	75.7 A	209.6	8.33	70.2	35.9 A	209.3 B	115.0 B	1.39	4.14 B
	Vinjett	58.4 B	215.6	8.25	69.5	33.3 B	237.5 A	115.6 A	1.38	4.73 A
Irrigation	Well-watered	74. 0 A	211.2	8.19	72.3 A	35.3	234.8 A	116.8 A	1.37	4.46
	Drought stress	60.1 B	214.1	8.38	67.4 B	33.9	211.9 B	113.8 B	1.40	4.40

Mean values followed by ± SE for different treatment combinations are given. Value sharing different letters within a column for a particular main effect indicate significant difference within climate, cultivar and irrigation treatments ($P < 0.05$). GLA, green leaf area; SLA, specific leaf area; GY, grain yield; TGW, thousand grain weight; TAGB, total above ground biomass; PM, physiological maturity; $WUE_{GY,}$ whole plant water-use efficiency on the basis of grain yield and; WUE_{TY} on the basis of total biomass basis. Normal, hot humid and hot dry are three climate treatments. Taifun and Vinjett are two cultivars. WW and DS are well-watered and drought stress treatments, respectively.

(Table 3). Lobell et al. (2013) showed a negative correlation between days with high VPD and grain yield of rainfed and associated it with water stress with no direct heat effects. Lack of VPD effect on grain yield in our study could be because, unlike Lobell et al. (2013), we studied irrigated wheat, where water was applied under high VPD and indirect effects VPD on drought were minimized. Secondly, model-based analysis and models usually do not account for the compensatory processes under stress, i.e. acclimation and tolerance. Perhaps due to similar reasons, Sánchez-Díaz et al. (2002) reported an increase in grain yield of barley under relatively high VPD and concluded that the combined effects of high temperature and drought were mitigated by high VPD. Others have associated the positive effect of VPD on grain yield with solar radiation (i.e. greater solar radiation on days with high VPD) (Lobell, 2007; Roberts et al., 2012). The effect of heat stress alone (HH climate) on green leaf area was more severe for Vinjett than Taifun cultivar, however, no yield difference was observed between the two cultivars.

Maintaining GLA and remobilization of assimilates to grains have been reported to be important stress tolerance mechanisms (Tahir and Nakata, 2005; Vignjevic et al., 2015). We used spike weight ratio as a proxy to estimate the assimilate allocation to grains, which was relatively higher under high VPD than low VPD at 36° C, indicating an increased grainfilling rate under high VPD. This resulted in higher grain weight, not only after the stress period (Fig. 6) but also at final harvest (Table 3). Sánchez-Díaz et al. (2002) also reported positive impact of high VPD under high temperature on grain filling rate in barley. Similar effect of high and low VPD at 36° C on TAGB indicates that even with changes in the resource allocation within the plant organs, the overall net assimilation was not changed (TAGB after stress and at final harvest, Table 3). Hence, translocation of assimilates to grains was a key tolerance mechanism for minimizing the temperature and VPD effects on grain yield, but what is the source of assimilates for higher grain filling rate under HD? This question as well arises because the rate of net photosynthesis was also at par between HH and HD (Fig. 1a). The mobilisable assimilates were probably water soluble stem carbohydrates, as evidenced by Vignjevic et al. (2015). With increase in spike weight ratio, there was a reduction in stem weight ratio (Fig. 6). Relatively higher harvest index under HD than HH (data not shown) also confirmed the relocation of assimilates to spike/grains during stress. Consequently, despite reduction in GLA and grainfilling duration under HD, GY and TAGB were similar to HH climate and no additive negative effect of temperature and VPD was found.

In contrast to VPD, drought reduced grain yield by 7% over well-watered. This was probably because apart from reduction in grainfilling duration, there was 20% reduction in GLA which was not compensated by remobilization of assimilates. Furthermore, the effect of drought and drought severity may have been amplified by high VPD through accelerated evapotranspiration, as reported by Will et al. (2013) and Lobell et al. (2013).

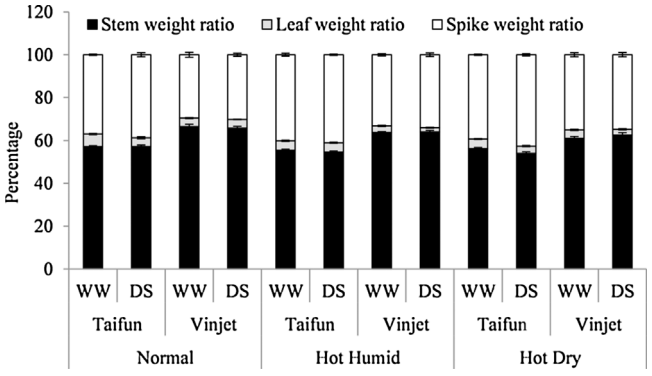

Fig. 6. Assimilate partitioning (leaf weight ratio, spike weight ratio, stem weight ratio) of wheat cultivars (Taifun and Vinjett) as affected by irrigation (well-watered, WW; drought stress, DS) and climate (normal: 24° C, 1.49 kPa VPD; hot humid: 36° C, 1.96 kPa VPD; and hot dry: 36° C, 3.92 kPa VPD). Measurements were done 3 days after the stress period. Error bars represent ± SE (n = 4).

4.2. Acclimation to temperature and VPD

High temperature and VPD both can reduce net assimilation through effects on biochemical and diffusive components of photosynthesis (Perdomo et al., 2016), and VPD above 2 kPa may even arrest crop growth by limiting transpiration (Fletcher et al., 2007; Rodriguez and Sadras, 2007), which would result in yield reduction. In this study, as an immediate effect after onset of the treatment, high VPD under high temperature (HD) reduced net photosynthesis (A_n) through stomatal (g_s) and non-stomatal limitations; however, on SD3 A_n under WW conditions recovered to the non-stress (24° C; 1.49 kPa) level. Positive change from SD1 to SD3 in photosynthesis under WW conditions occurred through acclimation of stomatal conductance, rubisco carboxylation efficiency (CEi) and photosynthetic apparatus, not only to high temperature but also VPD (Fig. 1a,b; Fig. 3S, Table 1S). Reduction in QC_i from SD1 to SD3 suggests acclimation of PSII because QC_i is a ratio of ΦPSII (operating quantum yield of PSII) to ΦCO_2 (quantum yield of CO_2 assimilation) (Oberhuber and Edwards, 1993), which are affected by PSII efficiency and CE_i. Thus, acclimation of A_n to higher temperature was probably related to recovery of rubisco carboxylation and PSII efficiencies.

Acclimation of non-stomatal components of photosynthesis (PSII efficiency) to high temperature have previously been shown by Haque et al. (2014) in similar conditions. Wheat acclimation to high temperature has also been used as a “priming technique” to minimize the negative effects of severe heat stress (Wang et al., 2012). However, acclimation of wheat photosynthetic components to high VPD as a separate factor has rarely been studied. Perdomo et al. (2016) reported acclimation of diffusive and biochemical components of photosynthesis in rice and maize at high temperature and VPD; however, they did not separate heat and VPD effects, and it remained unclear from their study whether the plants acclimatized to temperature or VPD. Acclimation of net assimilation to high temperature and VPD in present study ultimately resulted in minimizing the combined effects of high temperature and VPD on grain yield (Table 3).

4.3. Gas exchange and related physiological parameters

Inconsistent results have been reported for response of wheat gas exchange (A_n, g_s, T_r) to changes in VPD and drought (Xue et al., 2004). The results from the present study showed that A_n was reduced under high VPD, temperature and drought on SD1, but these effects disappeared on SD3. No difference in A_n for HH and HD on SD3 can be explained by similar SPAD, F_v/F_m and CE_i values (Fig. 3a, b, c). Although high temperature reduced PSII efficiency (F_v/F_m), the F_v/F_m values were never lower than 0.78 which is almost the lower limit of non-stressed plants (Maxwell and Johnson, 2000). Therefore, on SD3 neither temperature nor VPD affected the assimilation rate.

The effect of VPD on A_n in wheat has been associated with soil moisture stress (Xue et al., 2004), but we observed a tendency of lower (though non-significant) A_n even under well-watered conditions which signifies the importance of VPD as an immediate effect during heat stress. In contrast with VPD, A_n was more sensitive to soil moisture on all stress days which is consistent with Xue et al. (2004) who reported a decrease in A_n even under moderate drought stress. Effect of DS on A_n was mediated by lower g_s and CE_i (Figs. 1 b and 3 c) and higher instantaneous quantum cost (Fig. 2c).

VPD can also limit A_n through effects on stomatal conductance (g_s); however, g_s responses to VPD and response mechanism is not clearly understood (McAdam and Brodribb, 2015). In this study, high VPD (HD) reduced g_s but it remained unclear whether g_s responded to VPD or relative humidity (RH). This is because required levels of VPD were

reached by changing RH at same temperature (36 °C). The curvilinear response of g_s to increasing VPD (NC to HD) in this study was consistent with previous studies (Eamus et al., 2008; Kröber and Bruelheide, 2014; Kröber et al., 2015; Li and Li, 2014; Xue et al., 2004) who also did not consider RH. Different response mechanisms have been proposed in past, including the feedback mechanism, which means VPD induced increase in T_r causes reduction in g_s (Gourdji et al., 2013), and feed-forward mechanism suggesting that the controlling factor or signal is exclusively external, i.e. VPD (Bunce, 1997). Nonetheless, comparatively lower g_s even with high T_r under high VPD (HD) in our study contradict the feedback mechanism and hints to a direct VPD effect on g_s. The effect of VPD on g_s was consistent for the two cultivars under both irrigation regimes (Fig. 1b), which aligns with the findings of Xue et al. (2004) who reported g_s sensitivity to VPD irrespective of soil moisture content.

The major indirect effect of high temperature through increased VPD is increased crop water demand (evapotranspiration; ET) (Lobell et al., 2013; Will et al., 2013). However, transpiration response to increasing VPD is cultivar dependent and could be linear or curvilinear (Schoppach et al., 2014; Schoppach and Sadok, 2012; Schoppach and Sadok, 2013). Our study showed that as an immediate effect, high VPD at high temperature clearly increased transpiration (Fig. 1c). The difference in T_r between HH and HD declined over time, mainly because of stomatal conductance, which was not only higher under HH but also increased more under low than high VPD, followed by increase in transpiration. This suggests that stomata tend to adapt to changes in VPD more easily when it's within a certain limit and the limit may vary for different cultivars (Xue et al., 2004). It's known that for a given DS level, the reduction in actual Tr is greater for a climate with higher potential T_r compared to a climate with lower potential Tr (Denmead and Shaw, 1962). In our study, the reduction in T_r under DS was higher for HD than under HH, therefore less water was applied to DS plants under HH. However, the amount of water application remained same during five days of stress; the increased Tr under HH over time might have changed the soil moisture level.

Tr response to increasing VPD (1.49–3.92 kPa: NC to HD) for the two cultivars and irrigation levels on SD3 agreed with reported findings of a linear increase in Tr with increasing VPD until certain point and after that it started to decrease (Schoppach et al. (2014); Schoppach and Sadok (2012); Schoppach and Sadok (2013); Xue et al. (2004)). A linear relation between transpiration and flag leaf cooling (Fig. 4a) denies the possible involvement of feedback effect of high transpiration on further transpiration as indicated by Gourdji et al. (2013).

Different transpiration rates may disturb plants tissue water status which needs to be maintained for plants to survive stress (McDowell et al., 2008). Despite different transpiration rates, we found similar Ψ_L under different climates when plants were well-watered (Fig. 5). However, effect of VPD was different under drought stress, where Ψ_L was lower under high than low VPD. This could be due to two reasons; 1) excessive evapotranspiraton under high VPD could have intensified the drought compared to under low VPD (Will et al., 2013), and 2) greater depletion of the plant water reservoir through higher transpiration compared to root water uptake resulting in low Ψ_L (Katerji et al. (1986), which emphasizes the role of plant-reservoir in explaining the lack of linearity between the Ψ_L and transpiration.

4.4. Leaf- and whole plant water-use efficiency

Our study did not confirm the indications of Lobell et al. (2013) and Will et al. (2013) that temperature induced increase in VPD would reduce crop growth through drought effects. If plants are not drought stressed, high VPD reduces water-use efficiency because water loss is enhanced with little direct positive effect on CO_2 uptake (Condon et al., 2002; Sadras and McDonald, 2012; Sánchez-Díaz et al., 2002). Therefore, high VPD decreased WUE_p (Fig. 2a); however, due to acclimation in transpiration rates over time, difference between low and high VPD decreased during the stress period until SD3. In contrast with WUE_p, high VPD increased WUE_i on SD3 because of a curvilinear decrease of stomatal conductance with rising VPD as discussed above under gas exchange and mentioned by Schoppach et al. (2014); Schoppach and Sadok (2012); Schoppach and Sadok (2013); Xue et al. (2004).

Despite some effects of VPD on leaf level water-use efficiency, effect of high VPD on whole plant water-use efficiencies (WUE_{TY} and WUE_{GY}) was not different from low VPD (Table 3). Furthermore, the combined effects of high VPD and heat stress were not additive, as hypothesized; in fact there was a tendency of higher WUE_{GY} under high VPD compared to low VPD (heat effects alone). High VPD increased grain yield (Table 3) in accordance with findings of Sánchez-Díaz et al. (2002) who also indicated alleviating role of VPD under heat and drought stress.

Drought on the other hand improved WUE_p of Vinjett due to relatively high T_r under well-watered conditions and relatively high A_n under drought stress (Fig. 2a). Similarly, relatively greater reduction in g_s than in A_n improved WUE_i of Vinjett. The two cultivars therefore differed in their responses with respect to water-use efficiency under drought stress. However, while selecting crop cultivars for higher WUE, the relation between WUE_p and grain yield should be kept in mind. In our study, this relation was largely inverse for both cultivars under extreme climate conditions viz. relatively higher grain yield with lower WUE_p (Fig. 2a, Table 3). Such relation mainly exists for cereals, where high WUE_p is associated with slower crop growth; however contrary to this, wheat cultivars selected for high WUE_p showed no yield penalty across a range of environments in Australia (Condon et al., 2004). Wheat cultivars with high WUE_p can be opted if it's combined with high early vigor i.e. higher specific leaf area (SLA) (Condon et al., 2004), as it was observed in current study where Vinjett had relatively higher WUE_p and SLA under DS compared to Taifun but with no significant yield difference between the two cultivars. This suggests that both cultivars were equally efficient under DS but with different underlying mechanisms for maintaining the yield. Regardless of its effects on leaf level WUE, drought did not influence the whole plant WUE. Two possible reasons could explain this 1) the response of GY and TABG to water-use was linear 2) drought stress was probably not very severe and the stress period only lasted 5 days.

4.5. Implications and perspectives

Results imply that the deleterious effect of high temperature through increased VPD can be minimized by ensuring sufficient soil moisture supply; however, under rainfed conditions with terminal drought where irrigation is not possible, isohydric cultivars could be opted, because isohydric cultivars tends to close their stomata and maintain plant water status (Polania et al., 2016). Better understanding of direct and indirect effects of high temperature can also help to adopt, select or breed suitable crop cultivars i.e. drought or heat tolerant. Furthermore, temperature effects via heat stress can be mitigated by breeding crop cultivars with greater tolerance and acclimation potential. Crop modelling is the most common approach to study the impacts of climate change, but most models do not account for acclimation and tolerance potential of crops, therefore leading to uncertain results (Asseng et al., 2015; Cammarano et al., 2016). The present study results emphasize the need to improve crop models by taking into account some of the acclimation processes and physiological feedbacks.

We investigated the effects of a five-day stress event during post-anthesis stage; however, under climate change, plants may have to suffer from longer and more frequent stress episodes during different developmental stages. Future studies should examine how plants respond to longer and/or multiple stress episodes of high temperature and VPD at different developmental stages.

5. Conclusions

Temperature effects through VPD were mainly observed on crop

water-use. High VPD under heat stress increased leaf transpiration and decreased photosynthetic water-use efficiency. Transpiration rates varied during stress period and increased over time. VPD effects did not translate into yield loss. Combined effects of high temperature and high VPD under WW conditions on grain yield were minimized because of (a) acclimation of photosynthesis, stomatal conductance and carboxylation efficiency of rubisco to high temperature and VPD and (b) tolerance mechanisms which included maintaining the green leaf area and translocation of assimilates from stem and leaves to grains. Five days of high temperature- and drought stress reduced grain yield largely by affecting the green leaf area, grainfilling duration and grain weight.

Conflicts of interest

None

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agrformet.2017.09.018.

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